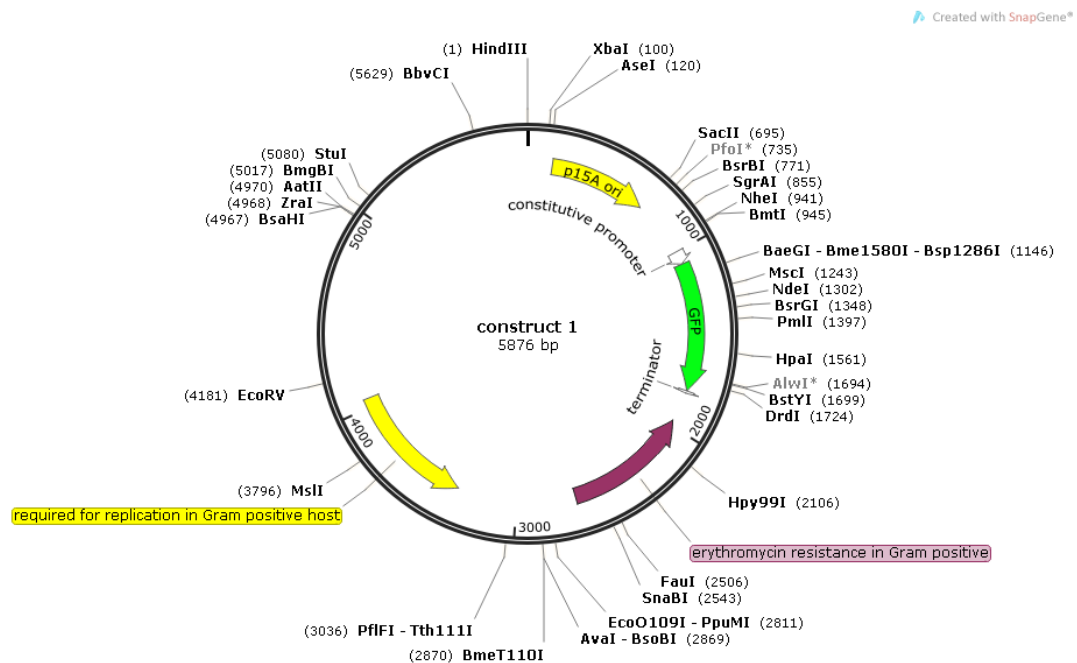


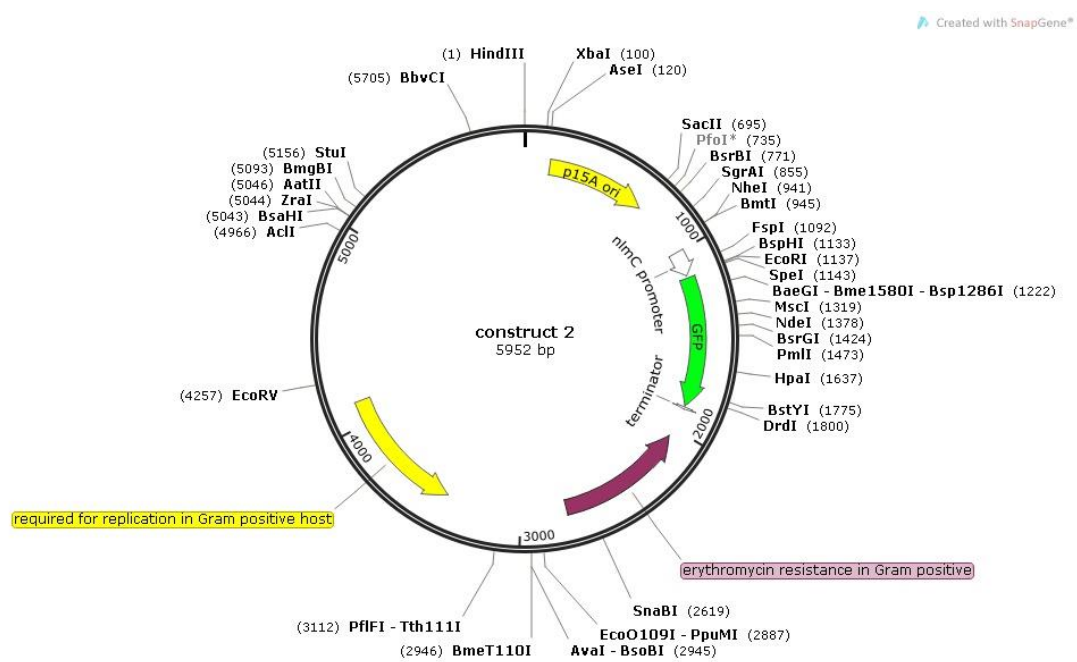
Lab note of target 1-1

Circuit Overview

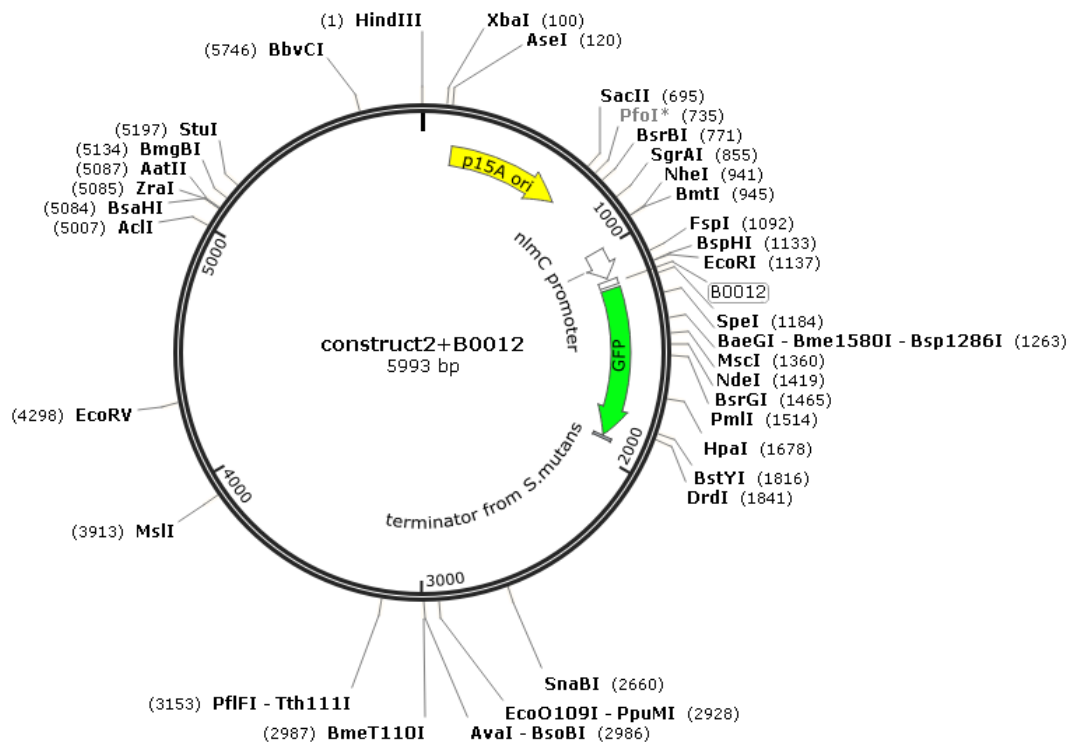
Construct1



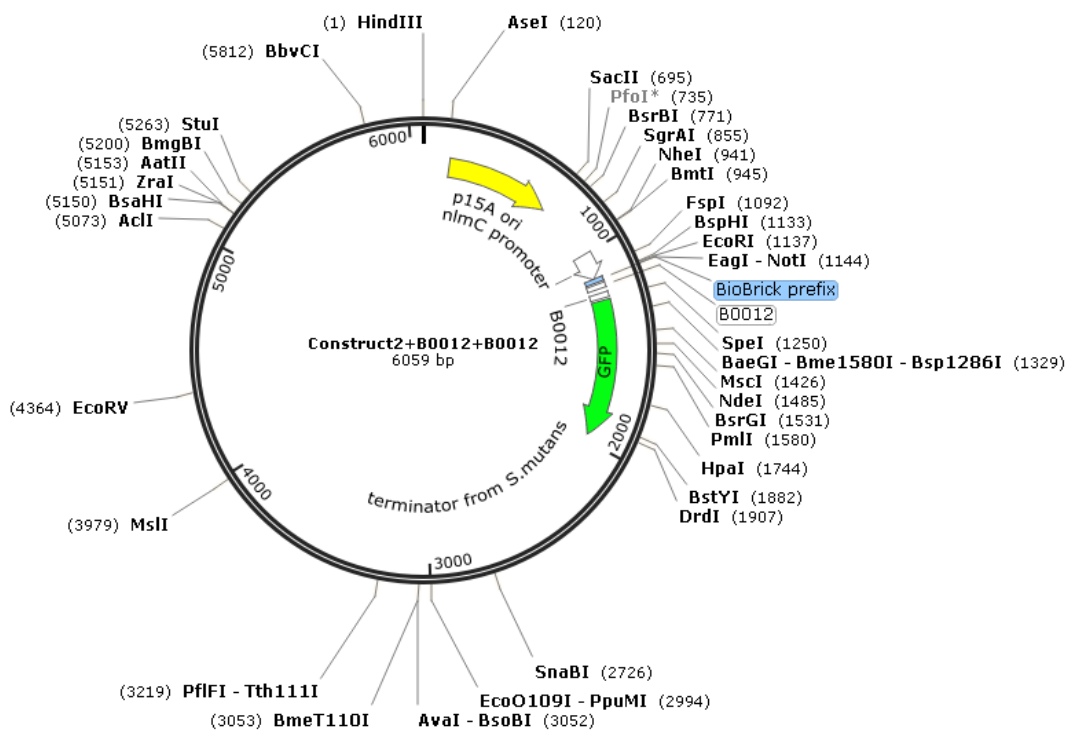
Construct2



Construct 2 + B0012



Construct 2+B0012+B0012



Lab Note

2014/07/25

pVA838 plasmid extraction

	ng/lambda	260/280
pVA838 plasmid	116.86	1.13

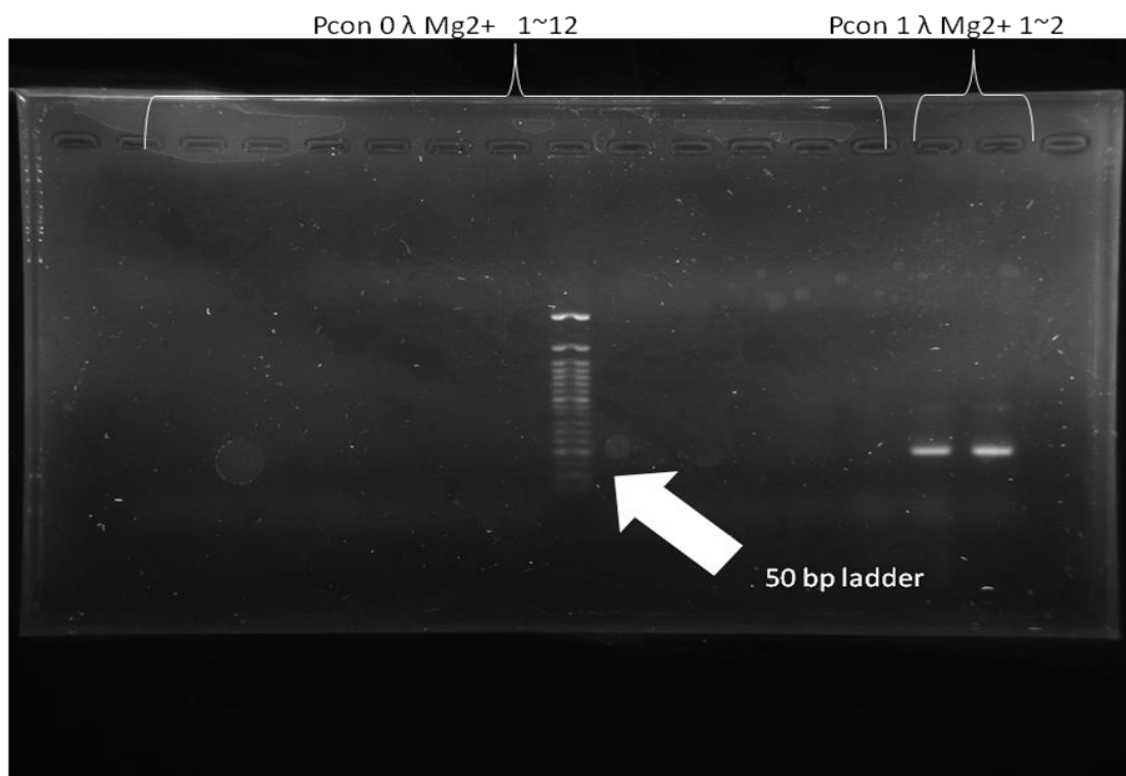
1. QC check:EcoR1 digestion
2. Electrophoresis: OK

2014/7/30

1. PCR and Electrophoresis of “constitutive promoter” and “ nlmC promoter”
 - (1) PCR
 - (2) Electrophoresis: fail
2. iGEM kit transformation: E0040, B0010, B0012, B0015

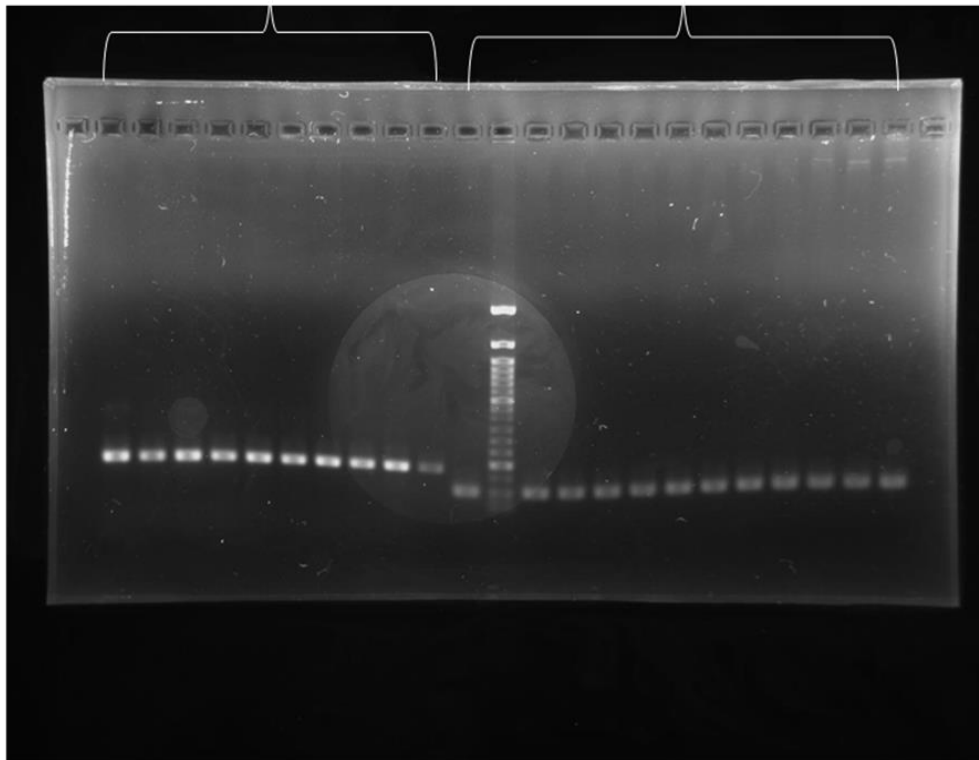
2014/7/31

1. PCR and Electrophoresis of “constitutive promoter” and “ nlmC promoter”
 - (1) PCR
 - (2) Electrophoresis

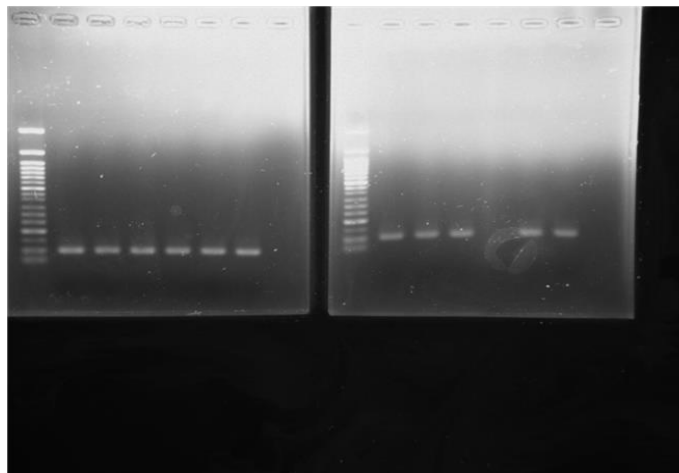


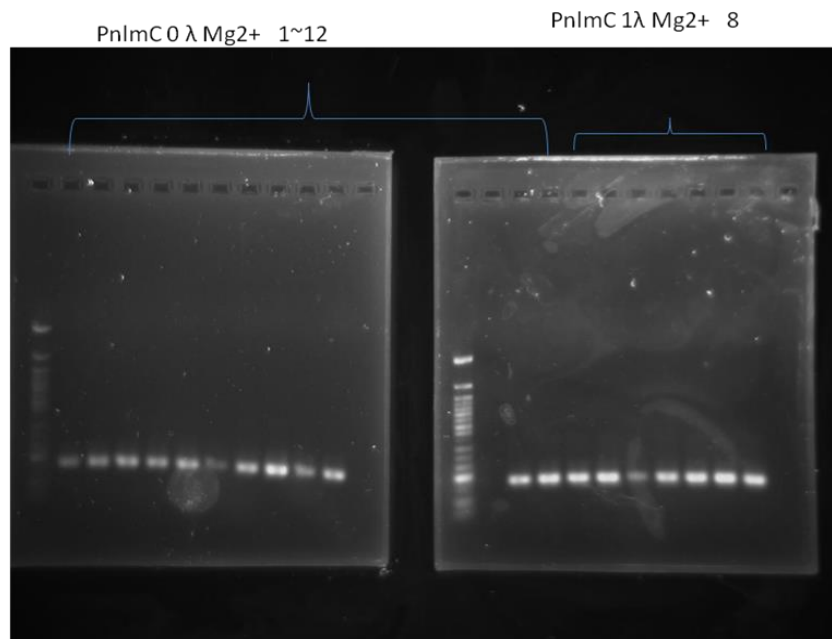
Pcon 1 λ Mg²⁺ 3~12

Pcon 2 λ Mg²⁺ 1~12



Pcon 4 λ Mg²⁺ 1~12





2014/8/1

1. PCR and electrophoresis iGEM kit: E0040, B0010, B0012, B0015

→ Fail

2. Transformation: B0010, B0012, B0015, E0040, E1010

2014/8/8

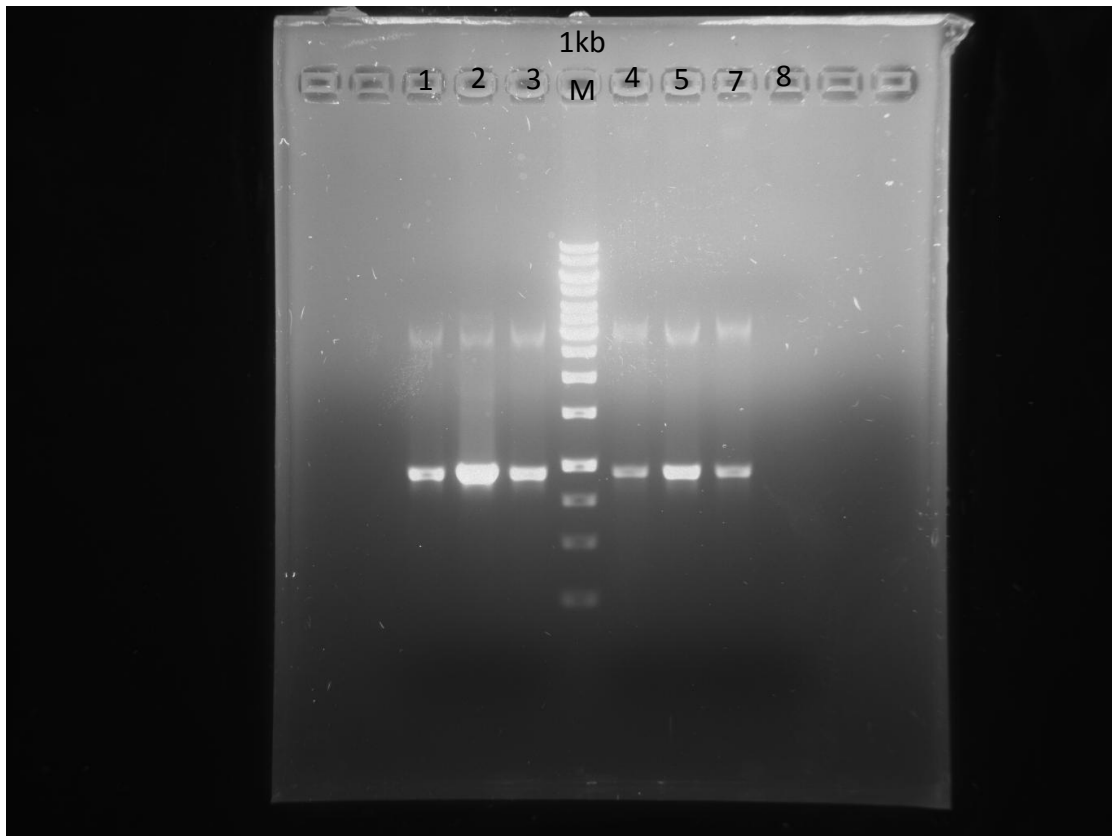
1. Colony PCR E0040 → OK
2. 2nd time plate+ liquid culture E0040

2014/8/9

1. Plasmid Extraction E0040

Sample ID	ng/ λ	260/280
E0040-1	86.31	0.99
E0040-2	181.52	1.26
E0040-3	123.81	1.14
E0040-4	143.61	1.19
E0040-5	146.48	1.19
E0040-7	136.52	1.16
E0040-8	122.81	1.13

2. Plasmid PCR E0040



2014/8/10

1. Transformation: B0010, B0012, B0015, E1010

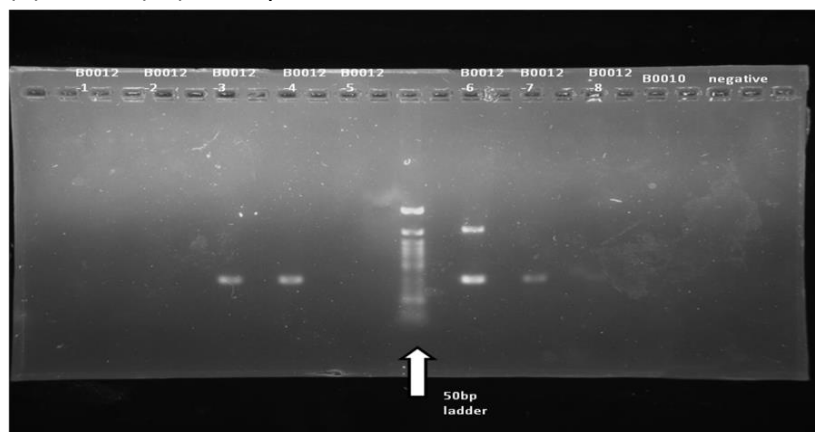
2014/8/11

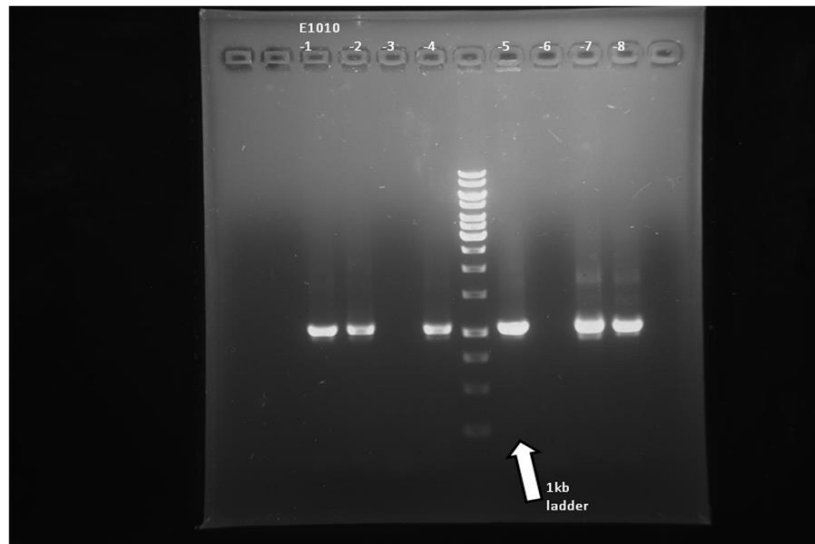
1. Colony PCR:

(1) E1010 (x8) 1000bp

(2) B0012(x8) 354 bp

(3) B0010(x1) 317 bp



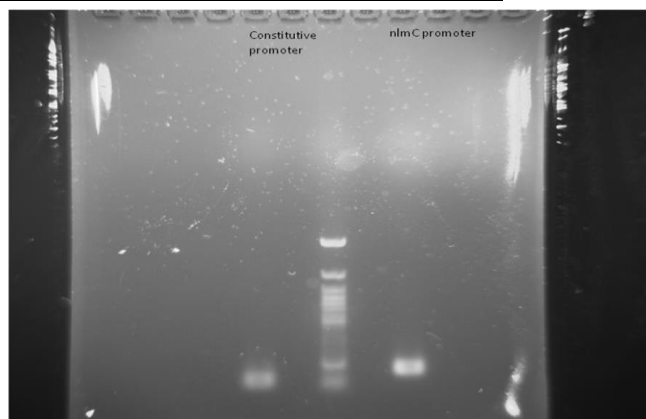


2. KOD PCR

(1) Constitutive promoter 104bp

(2) nlmC promoter 180 bp

Buffer	5
Primer	1
DNA(100ng)	1
KOD	0.8
Mg2+	2
dNTP	1
ddwater	41.2
total	50



3. liquid culture:

(1) E1010 (x6)

(2) B0012(x3)

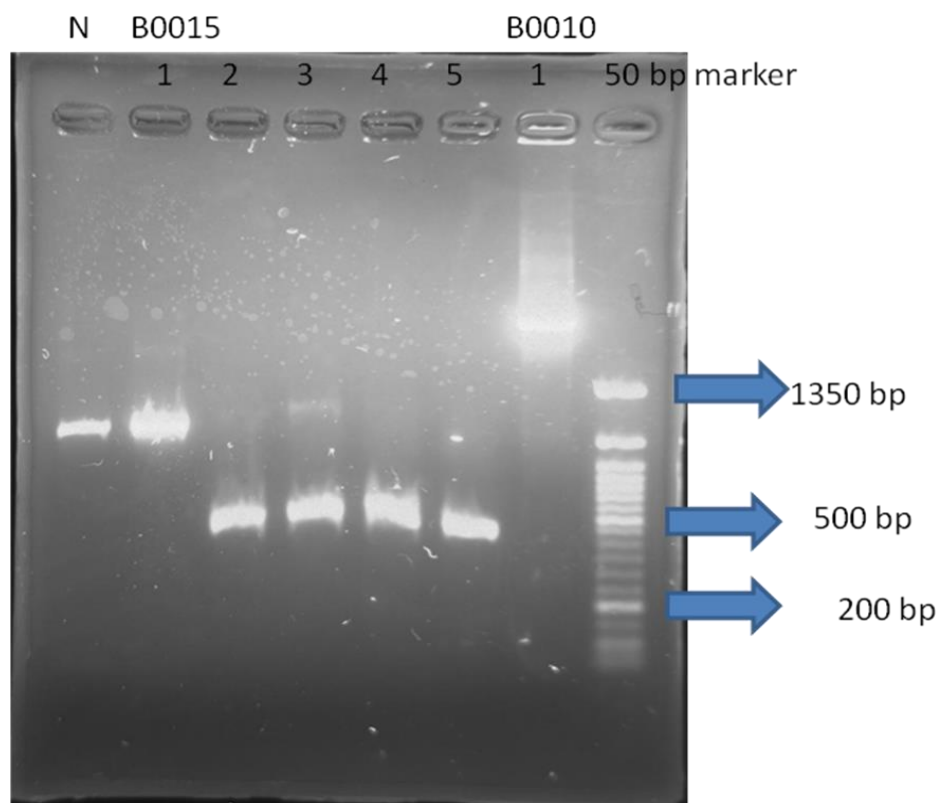
2014/8/12

1. Gel extraction: nlmC promoter, constitutive promoter

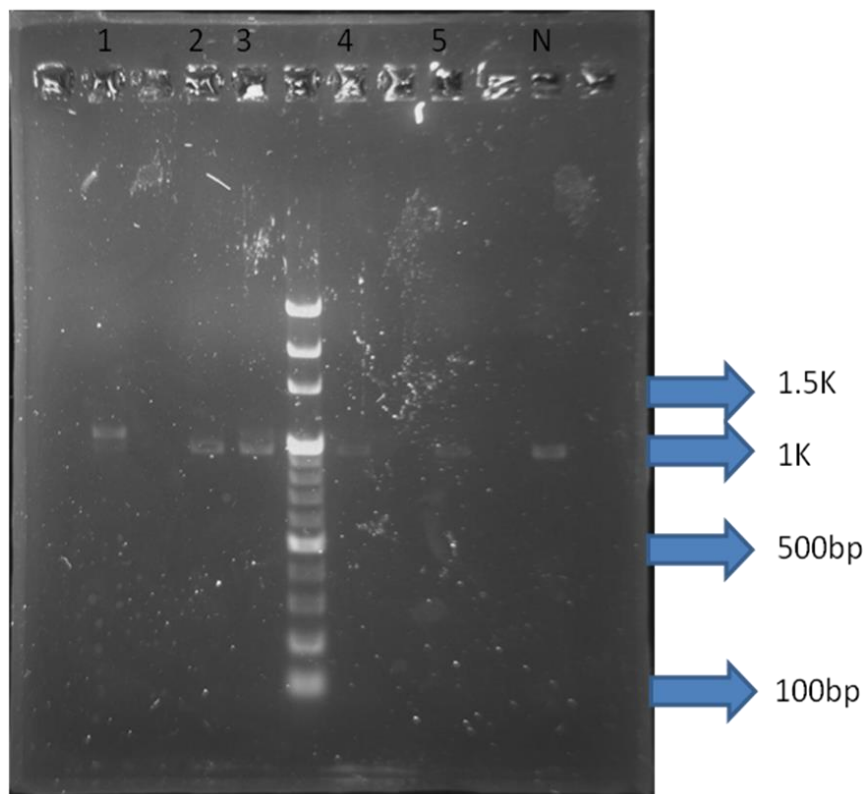
Sample ID	ng/ λ	260/280
-----------	---------------	---------

Constitutive promoter	29.33	0.51
nlmC promoter	36.67	0.61

2. B0015, B0010 colony PCR



B0010



3. Liquid culture: B0015(x4)

4. Plasmid extraction:

(1) E1010(1+2, 4+5, 7,8)

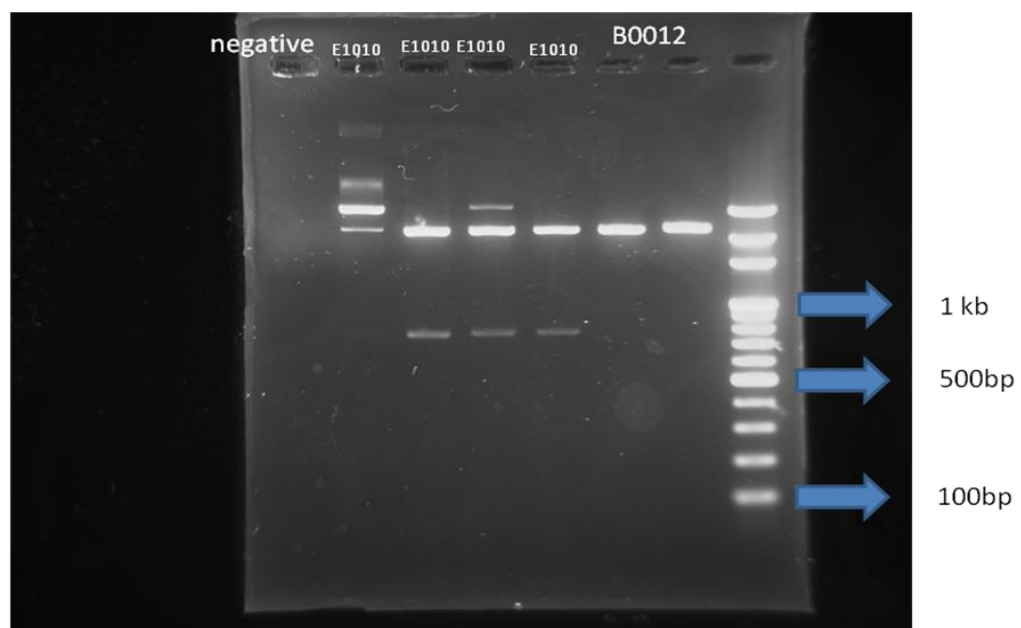
(2) B0012(3+4, 4+7)

Sample ID	ng/ λ	260/280
E1010(1+2)	172.74	1.25
E1010(4+5)	137.98	1.19
E1010(7)	84.63	1.01
E1010(8)	119.47	1.11
B0012(3+4)	114.45	1.07
B0012(4+7)	89.62	1.00

5. Digestion check: B0012, E1010

E1010: EcoR1& Pst1 (706&2070)

B0012: Xba1& Pst1(41&2070)



6. Liquid culture

(1) B0015(x4)

(2) B0010(x5)

2014/8/13

1. plasmid extraction

(1) B0015(x4)

(2) B0010(x5)

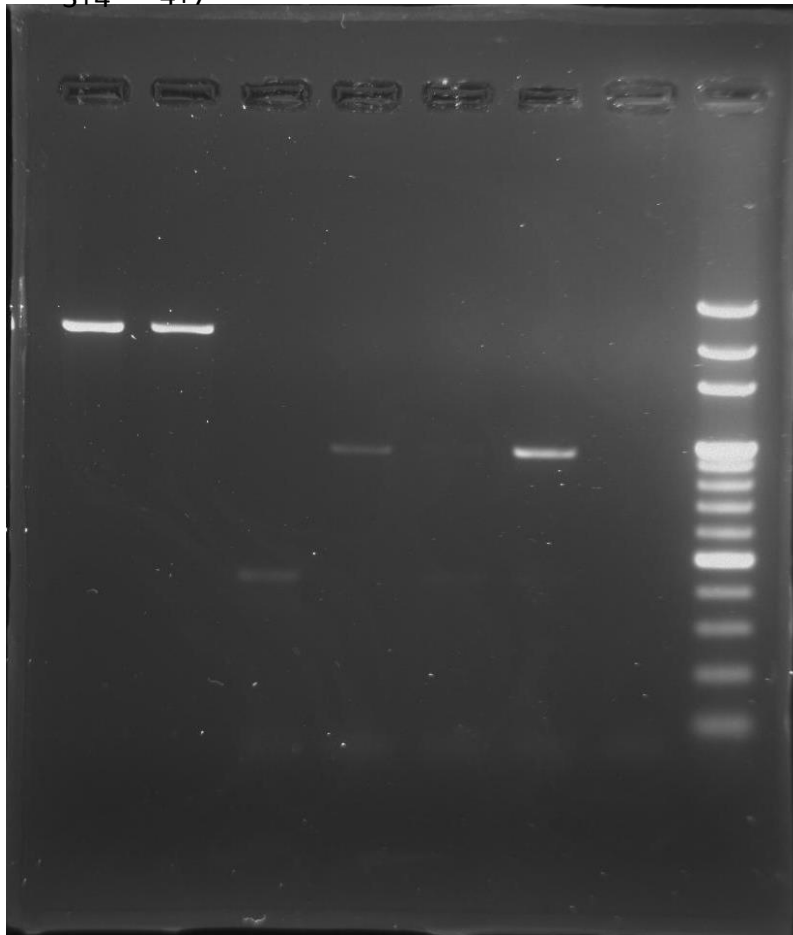
Sample ID	ng/ λ	260/280
-----------	---------------	---------

B0015-1	65.61	1.06
B0015-2	86.88	1.16
B0015-3	60.12	1.03
B0015-4	84.44	1.17
B0010-1	75.56	1.19
B0010-2	287.73	1.46
B0010-3	228.43	1.41
B0010-4	216.33	1.49
B0010-5	187.02	1.44

*Find red pellet in B0010(-2,-3,-5)

2. digestion check

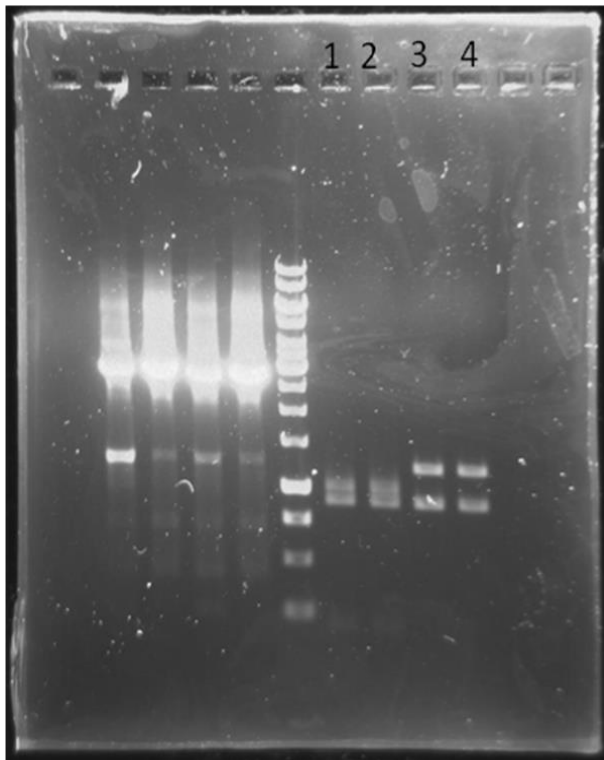
B0012 B0012
(1) B0012(3+4, 4+7)(Xba1&Pst1)
3+4 4+7



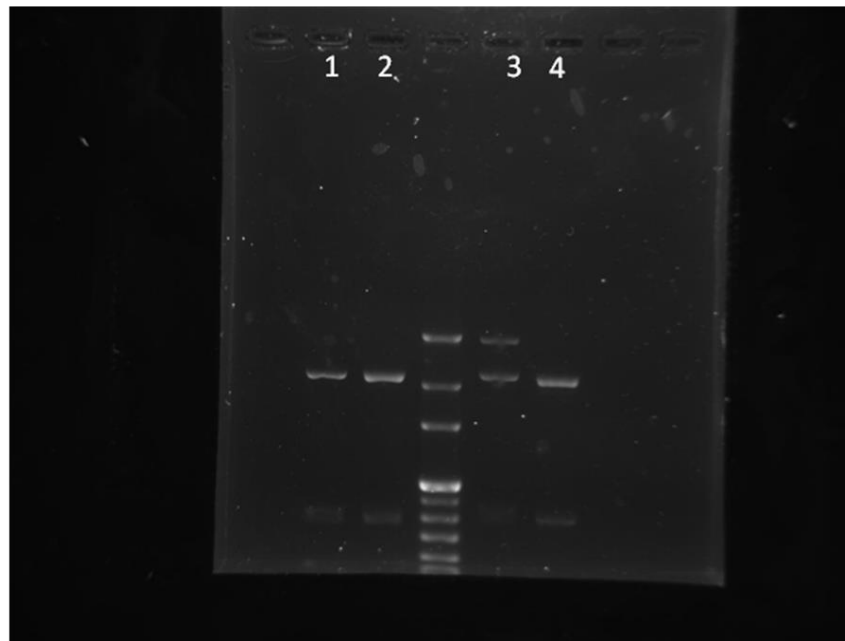
(2)B0012 digestion check

1,2= Xho1&Pst1(1014 bp &893bp&207bp)

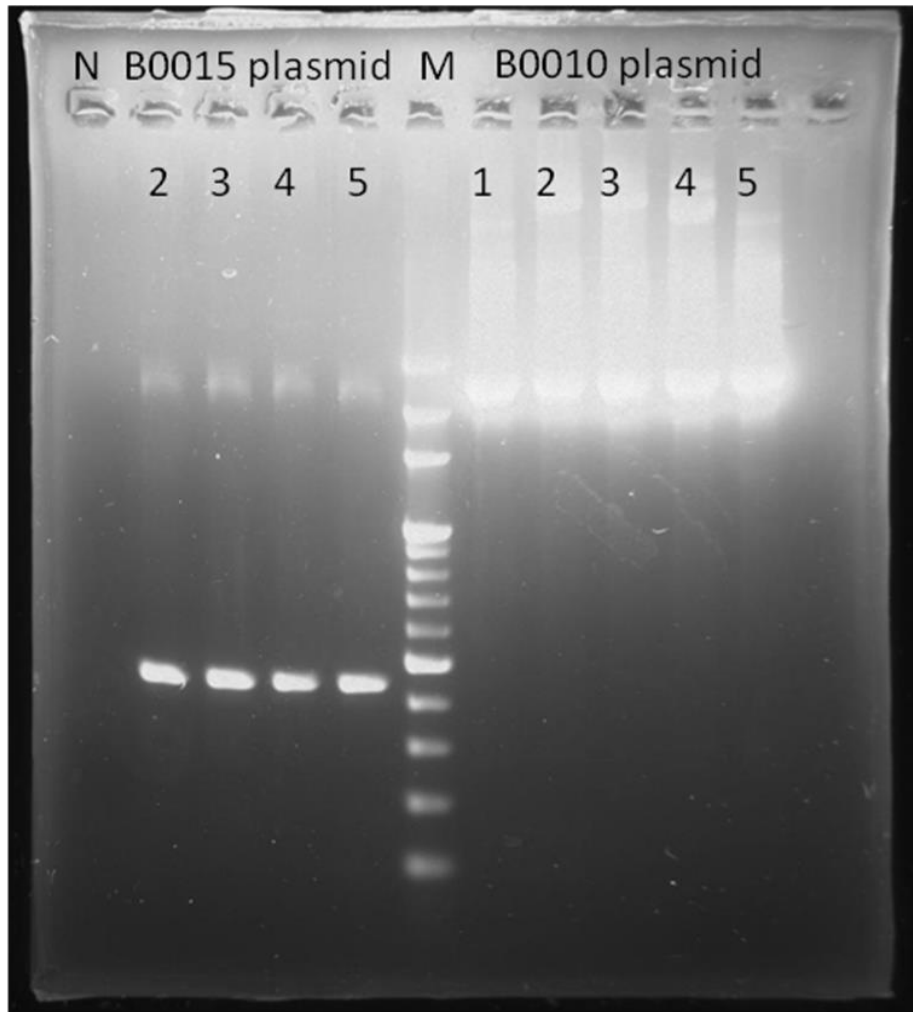
3,4=Xho1 (892bp&1219bp)



(3) E0040 digestion check(Xba1 + Pst1)
Result: correct: 2044bp & 746bp



(4) B0015, B0010 plasmid PCR
Result: B0015 (433bp)= correct
B0010 (218bp)= incorrect



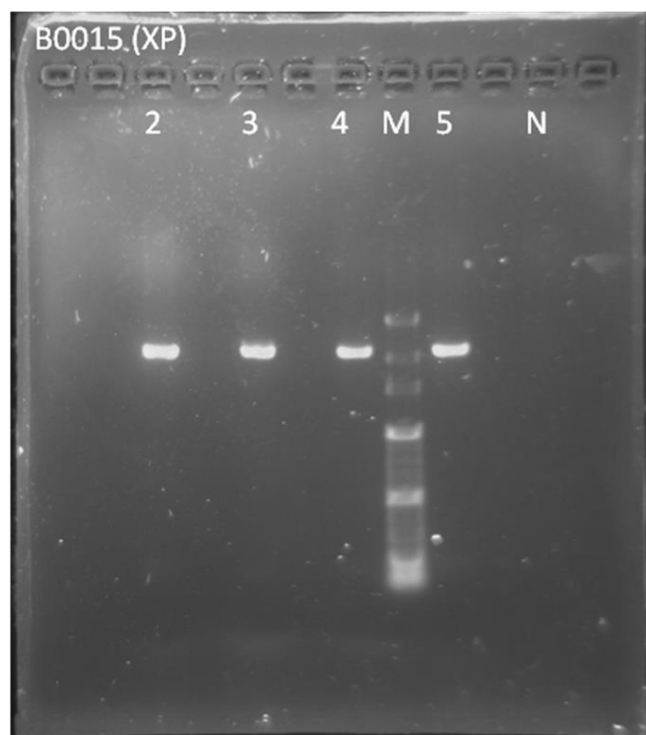
2014/8/14

(1) B0015 digestion check

Xba1 & Pst1= 129bp

&2070bp

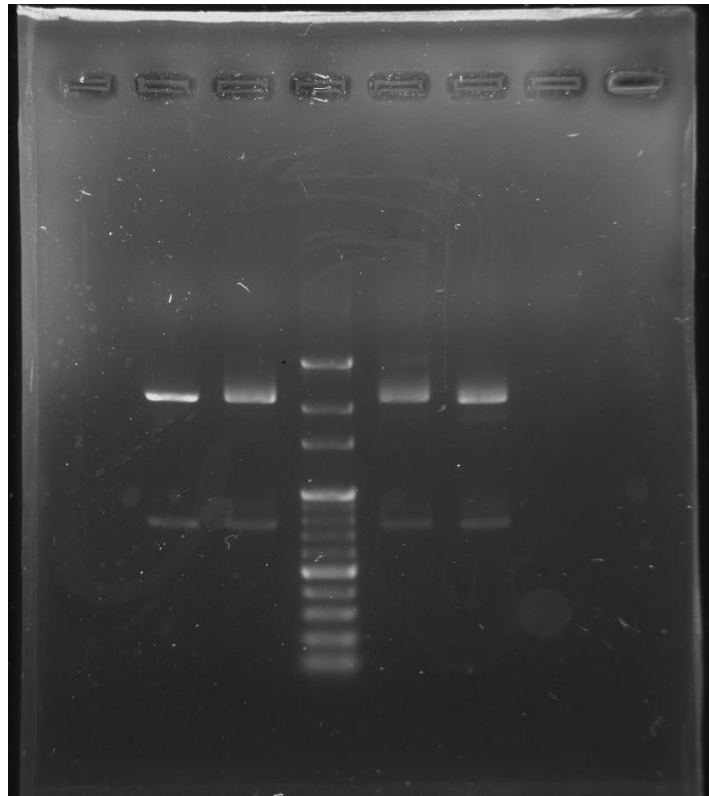
Result: all correct



(2) E0040 digestion check

Xba1&Pst1=2044bp &746bp

Result: correct

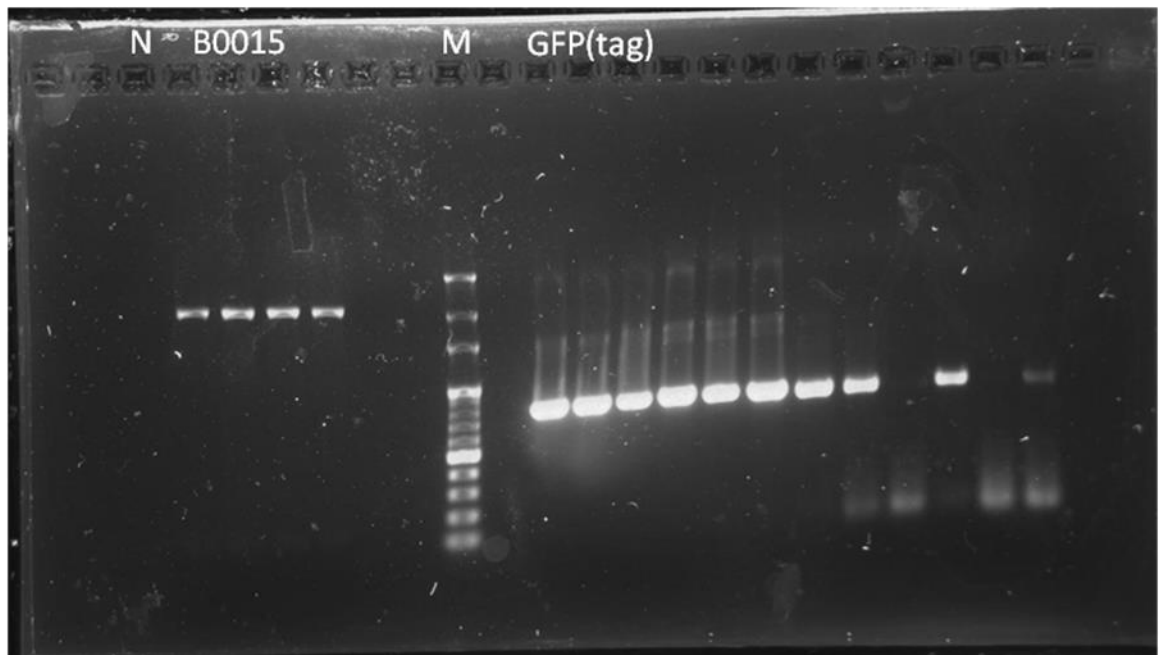


(3) GFP (1,2,3= after constitutive promoter ;4,5,6= after nlmC promoter) tag PCR

	1	N1	2	N2	3	N3
Template	0.11	0	0.11	0	0.11	0
Primer	0.2	0.2	0.2	0.2	0.2	0.2
dNTP	0.2	0.2	0.2	0.2	0.2	0.2
10xbuffer	1	1	1	1	1	1
tag	0.04	0.04	0.04	0.04	0.04	0.04
MgSO ₄	0	0	0.2	0.2	0.4	0.4
ddH ₂ O	8.46	8.56	8.16	8.36	8.06	8.16
total	10	10	10	10	10	10

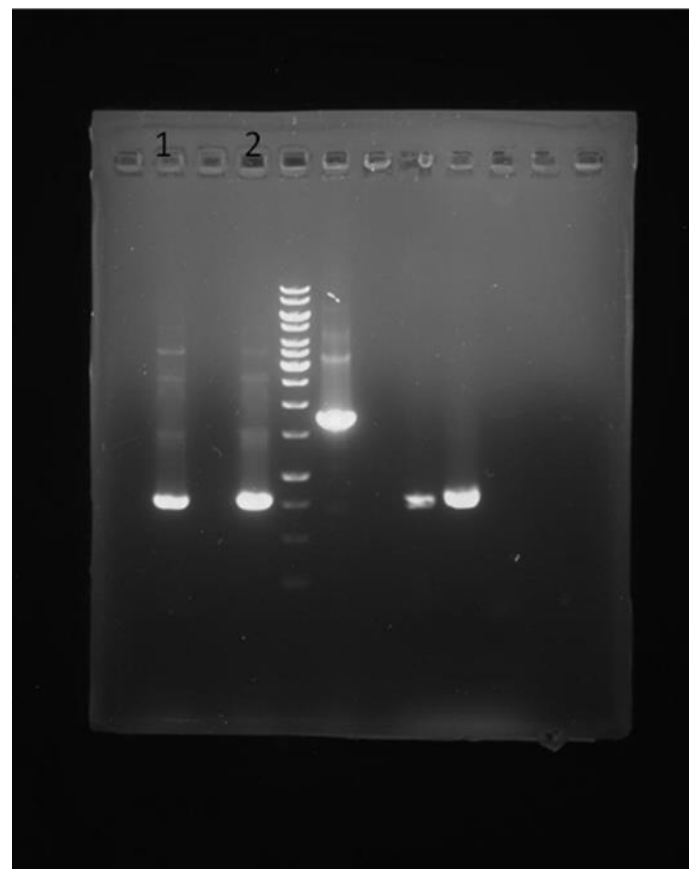
	4	N4	5	N5	6	N6
Template	0.11	0	0.11	0	0.11	0
Primer	0.2	0.2	0.2	0.2	0.2	0.2
dNTP	0.2	0.2	0.2	0.2	0.2	0.2
10xbuffer	1	1	1	1	1	1
tag	0.04	0.04	0.04	0.04	0.04	0.04
MgSO ₄	0	0	0.2	0.2	0.4	0.4
ddH ₂ O	8.46	8.56	8.16	8.36	8.06	8.16
total	10	10	10	10	10	10

- (4) B0015 digestion & GFP(after constitutive promoter & nlmC promoter) tag PCR
B0015=2070 bp & 129bp
GFP= 717bp



2014/8/15

1. GFP(after constitutive promoter & nlmC promoter) KOD PCR
1= GFP after constitutive promoter,
717 bp
2= GFP after nlmC promoter, 717 bp



2014/8/16

1. Backbone-constitutive & Backbone-nlmC tag PCR

(1) Backbone-constitutive

	1	N1	2	N2	3	N3	4	N4
Template (pVA838)	0.17	0	0.17	0	0.17	0	0.17	0
Primer	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
dNTP	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
10X buffer	1	1	1	1	1	1	1	1
Tag	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
MgSO ₄	0	0	0.2	0.2	0.4	0.4	0.6	0.6
ddH ₂ O	8.39	8.56	8.19	8.36	7.99	8.16	7.79	7.96
total	10	10	10	10	10	10	10	10

(2) Backbone-nlmC

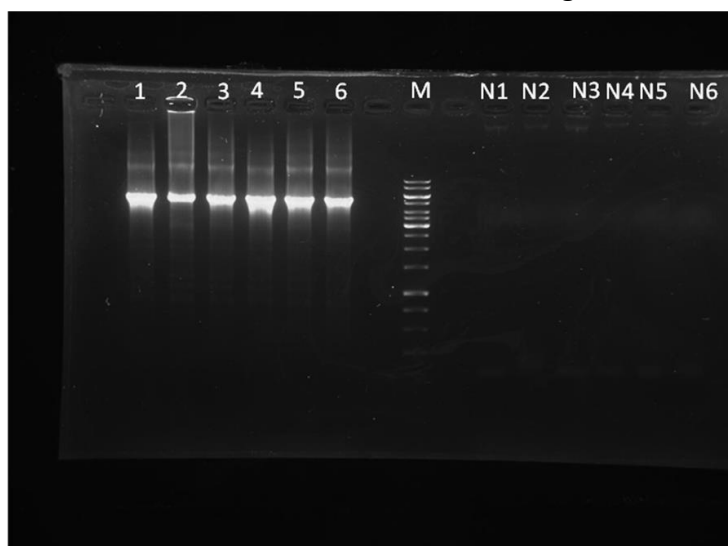
	5	N5	6	N6	7	N7	8	N8
Template (pVA838)	0.17	0	0.17	0	0.17	0	0.17	0
Primer	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
dNTP	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
10X buffer	1	1	1	1	1	1	1	1
Tag	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
MgSO ₄	0	0	0.2	0.2	0.4	0.4	0.6	0.6
ddH ₂ O	8.39	8.56	8.19	8.36	7.99	8.16	7.79	7.96
total	10	10	10	10	10	10	10	10

2. Electrophoresis of Backbone-constitutive, Backbone-nlmC tag PCR

5041bp

annealingTm:

58°C



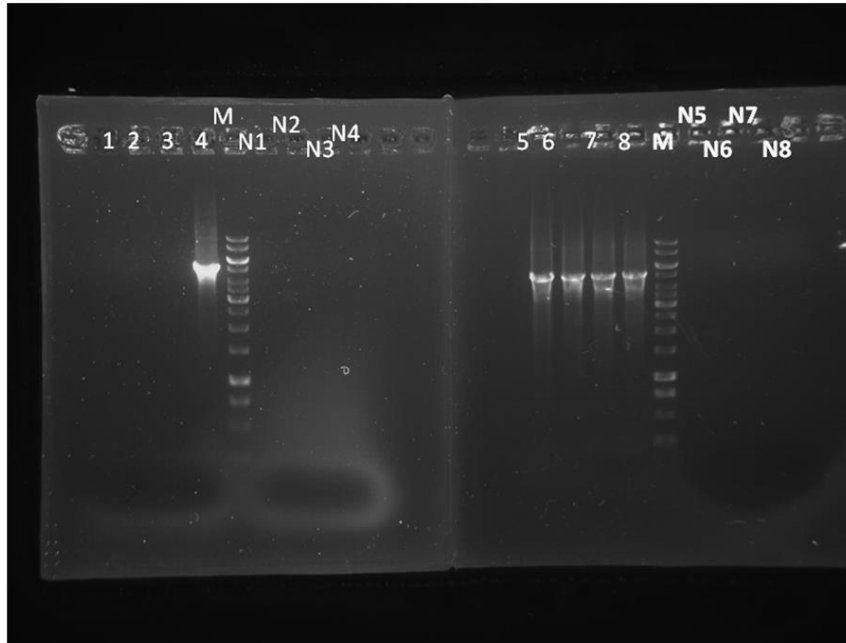
2014/8/18

1. Backbone-constitutive; Backbone-nlmC taq PCR

Annealing Tm: 60°C

*components of reagent are the same as the one done on August 18th, 2014

2. electrophoresis of Backbone-constitutive; Backbone-nlmC



2014/8/19

1. Backbone-constitutive, Backbone-nlmC KOD PCR

Annealing: 58°C

1,2= Backbone-constitutive

3,4=Backbone-nlmC

	1	N1	2	N2	3	N3	4	N4
Template	0.68	0	0.68	0	0.68	0	0.68	0
Primer	0.6	0.12	0.6	0.12	0.6	0.12	0.6	0.12
dNTP	1	0.2	1	0.2	1	0.2	1	0.2
10xbuffer	5	1	5	1	5	1	5	1
KOD	0.8	0.16	0.8	0.16	0.8	0.16	0.8	0.16
MgSO ₄	4	0.8	5	1	2	0.4	3	0.6
ddH ₂ O	37.92	7.72	36.92	7.52	39.92	8.12	38.92	7.92
total	10	10	10	10	10	10	10	10

2. Electrophoresis of Backbone-constitutive, Backbone-nlmC(KOD PCR)
3. Backbone-constitutive, Backbone-nlmC gel extraction

sample	ng/ λ	260/280
Backbone-constitutive1	55.06	0.7
Backbone-constitutive2	70.78	0.8

Backbone-nlmC 4	47.64	0.65
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2014/8/25

1. Gibson assembly

(1) Concentration of each part

Backbone-constitutive= 70.78 ng/ λ

Backbone-nlmC= 47.64 ng/ λ

Constitutive promoter= 29.83 ng/ λ

nlmC promoter=38.67 ng/ λ

GFP-constitutive=121.02 ng/ λ

GFP-nlmC=156.38 ng/ λ

(2) Dilution

Constitutive promoter= dilute to 1/20

nlmC promoter= dilute to 1/20

GFP-constitutive= dilute to 1/10

GFP-nlmC= dilute to 1/20

(3) Components of reagent

	constitutive	nlmC
Backbone	6	6
Promoter	0.5	0.5
GFP	1	1
Gibson assembly master mix (2x)	10	10
ddH ₂ O	2.5	2.5
total	20	20

2014/8/26 & 2014/8/27

1. Testing Erythromycin plate

Result= 125 mg/ml & 150 mg/ml are the most suitable

2014/8/28

1. Transformation of Construct 1(constitutive promoter+GFP)

Construct 2(nlmC promoter +GFP)

Construct 1: (1) 2 λ +35 λ competent cell

(2)8 λ +35 λ competent cell

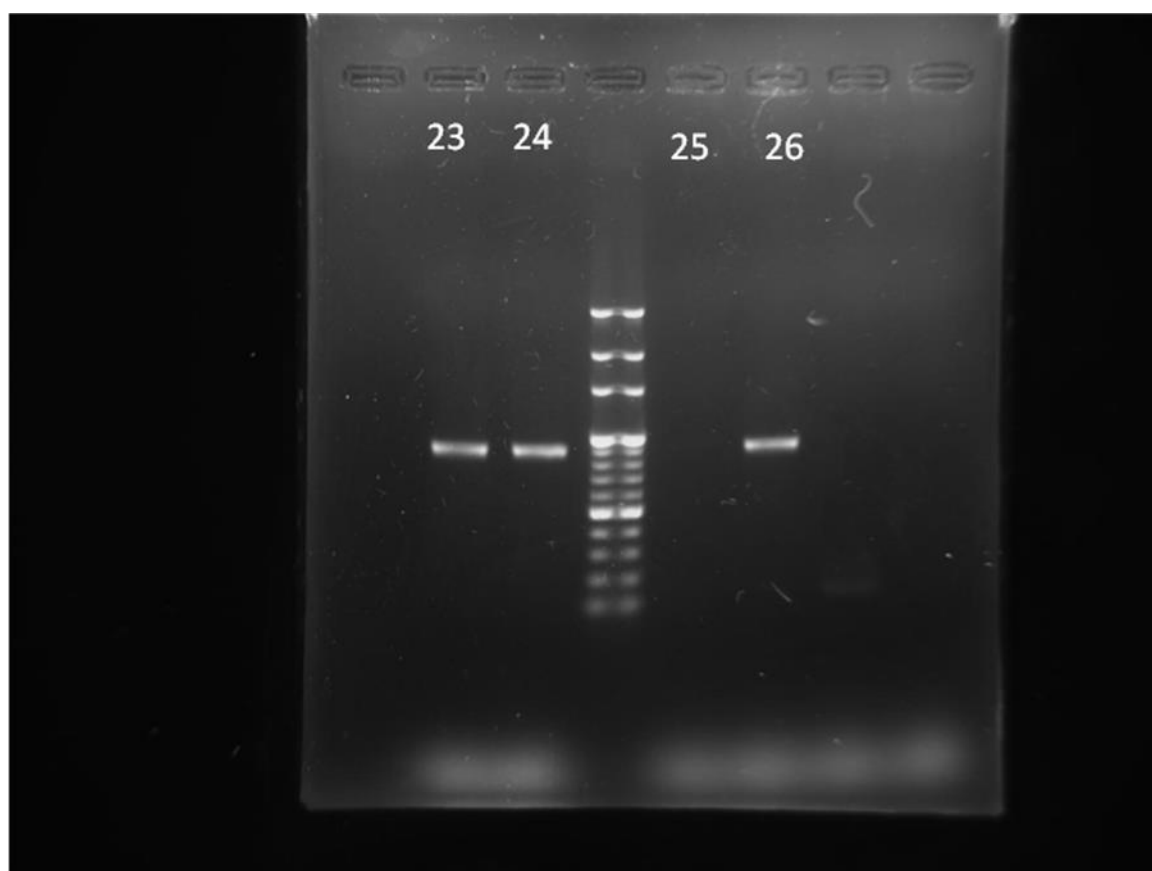
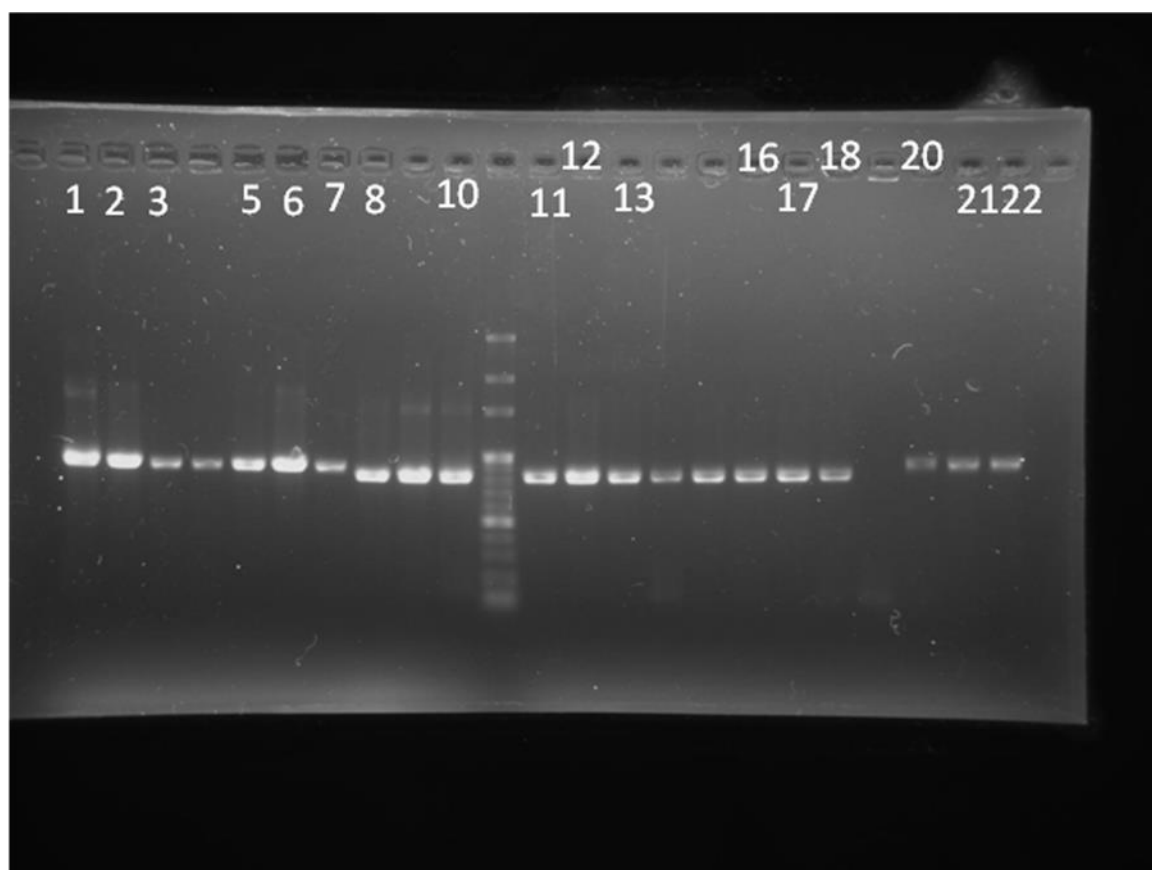
Construct 2: (1) 2 λ +35 λ competent cell

(2)8 λ +35 λ competent cell

Each transformation sample are plated out to 3 plates

2014/8/29

1. Colony PCR of construct 1&construct 2



2014/9/1

1. Plasmid extraction: construct 1 &2

sample	ng/ λ	260/280
1(construct2)	97.97	0.68
2(construct2)	171.52	0.93
3(construct2)	173.00	0.84
4(construct2)	165.33	0.90
5(construct2)	163.64	1.00
6(construct2)	208.65	0.87
7(construct2)	147.03	0.90
8(construct1)	166.67	0.79
9(construct1)	112	0.79
10(construct1)	124.65	0.72
11(construct1)	108.96	0.73
12(construct1)	110.84	0.77
13(construct1)	122.88	0.77
14(construct1)	126.89	0.78
15(construct1)	124.10	0.77
16(construct1)	116.52	0.75
17(construct1)	97.92	0.72
18(construct1)	125.40	0.77
19(construct1)	101.86	0.68
20(construct2)	142.91	0.82
21(construct2)	127.27	0.79
22(construct2)	241.83	1.06
23(construct2)	170.24	0.90
24(construct2)	149.05	0.87
26(construct2)	215.38	1.04

2. Digestion check (construct1 &construct2)

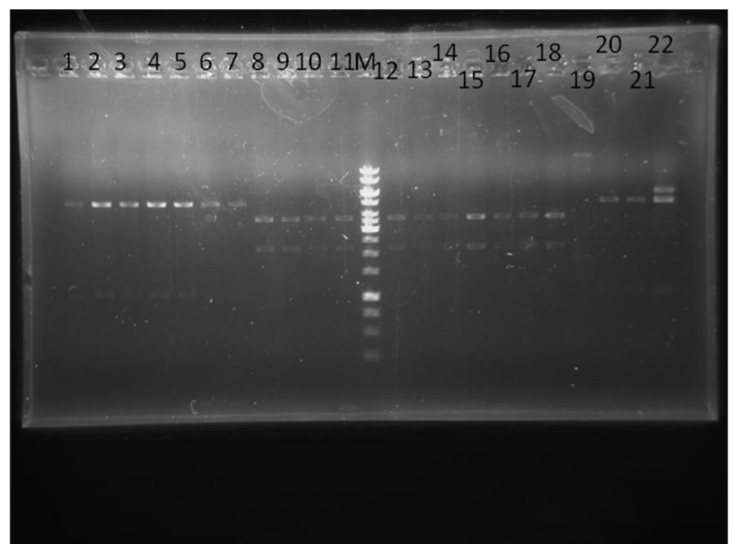
Construct1: 8~19 (Nco1; 2183bp&3693bp)

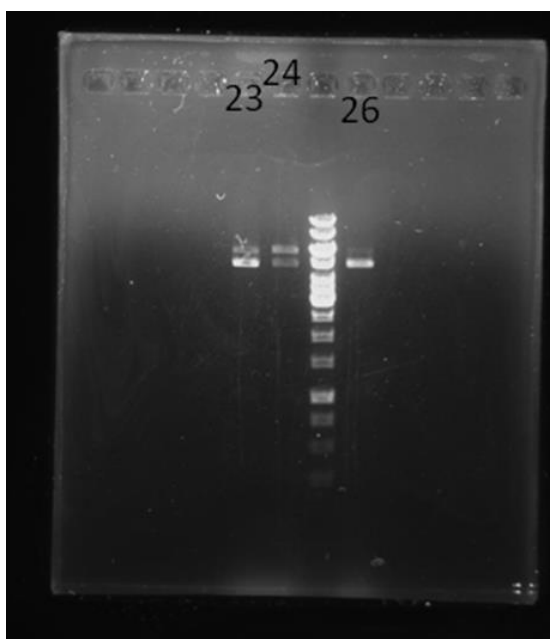
Construct2: 1~7; 20~26

(EcoR1&Xba1 1038bp&4914bp)

Result:

Correct:1~21





2014/9/8

1. B0012; B0015 digestion

- B0012(XP)+B0012(SP)
- B0015(XP)+B0012(SP)
- B0015(XP)+B0015(SP)

*Since Pst1 is used in FD buffer, while Spe1 is used in Cutsmart or NEB4+BSA buffer, B0012(SP) and B0015(SP) need to be digest twice.

(1) B0012(SP)&B0015(SP) Pst1 digestion

Sample	ng/ λ	260/280
B0012 (SP)-1	74.25	0.57
B0012 (SP)-2	74.85	0.57
B0015 (SP)	73.65	0.57

(2) B0012(SP)&B0015(SP) Spe1 digestion

(3) B0012(XP)&B0015(XP) Xba1&Pst1 digestion

Sample	ng/ λ	260/280
B0012(SP)-1 cutsmart	75.79	0.55
B0012(SP)-2 cutsmart	72.65	0.55
B0015(SP) cutsmart	73.41	0.56
B0012(SP)-1 BSA	74	0.56
B0012(SP)-2 BSA	73.08	0.55
B0015(SP) cutsmart	73.01	0.55
B0012(XP)	73.58	0.56
B0015(XP)-2	75.97	0.56

B0015(XP)-3	71.54	0.57
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2. Ligation

(1) B0012+B0012

(2) B0015+B0012

(3) B0015+B0015

2014/9/9

1. Transformation

(1) B0012+B0012

(2) B0015+B0012

(3) B0015+B0015

2. Ligation

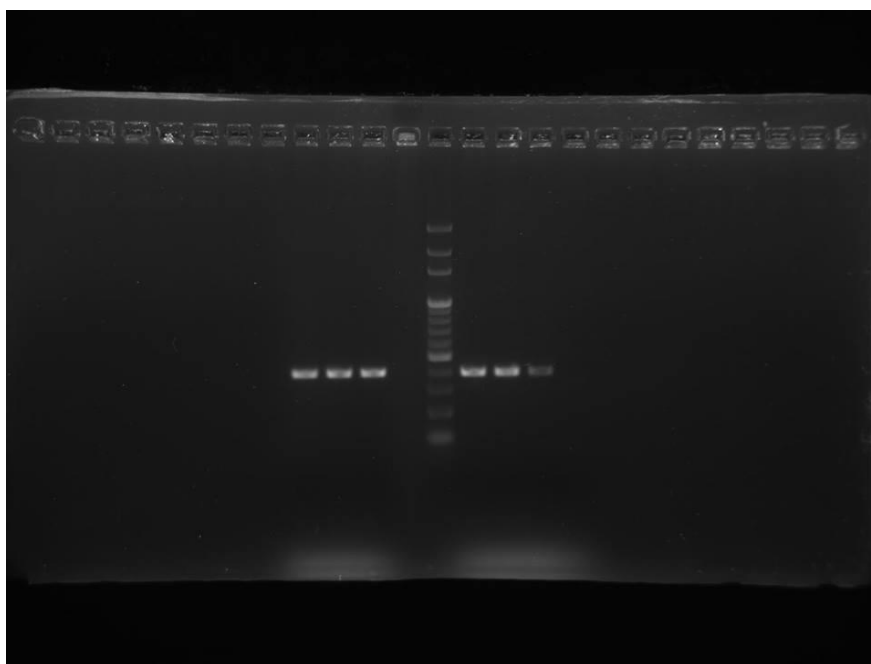
(1) B0015+B0012

(2) B0015+B0015

2014/9/11

1. Colony PCR (B0012+B0012)

406bp



2014/9/15

1. B0012+B0012 plasmid extraction

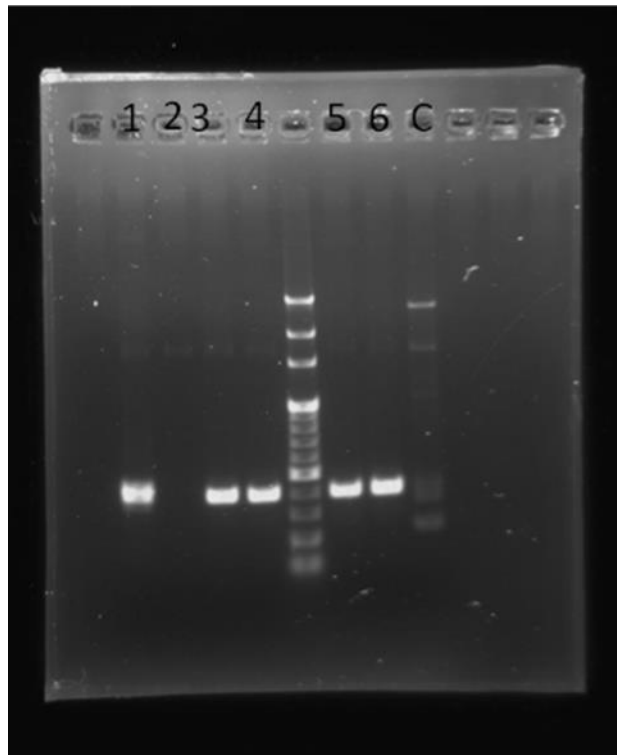
Sample	ng/ λ	260/280
B0012+B0012_1	40.30	0.85
B0012+B0012_2	51.61	0.94
B0012+B0012_3	45.02	0.89
B0012+B0012_4	38.20	0.82

B0012+B0012_5	58.52	0.87
B0012+B0012_6	60.78	1.00

2014/9/17

1. B0012+B0012 plasmid PCR

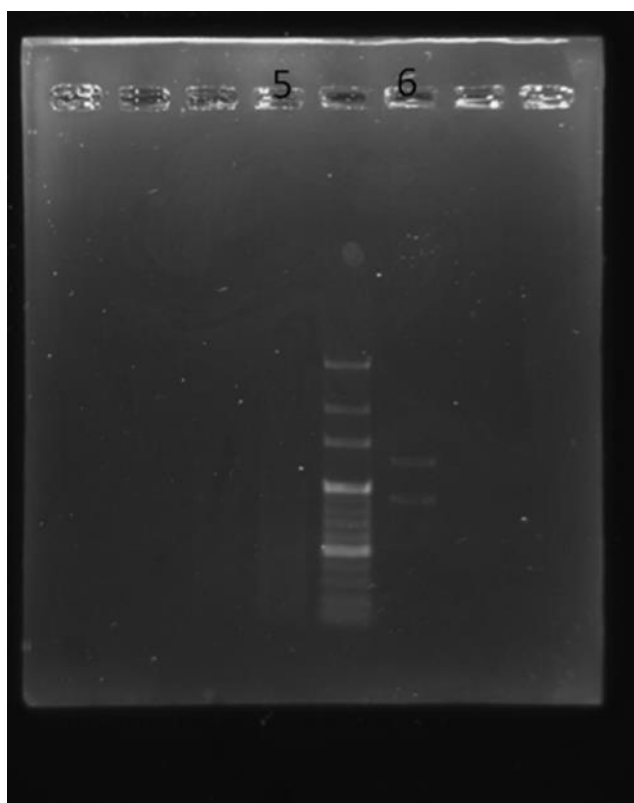
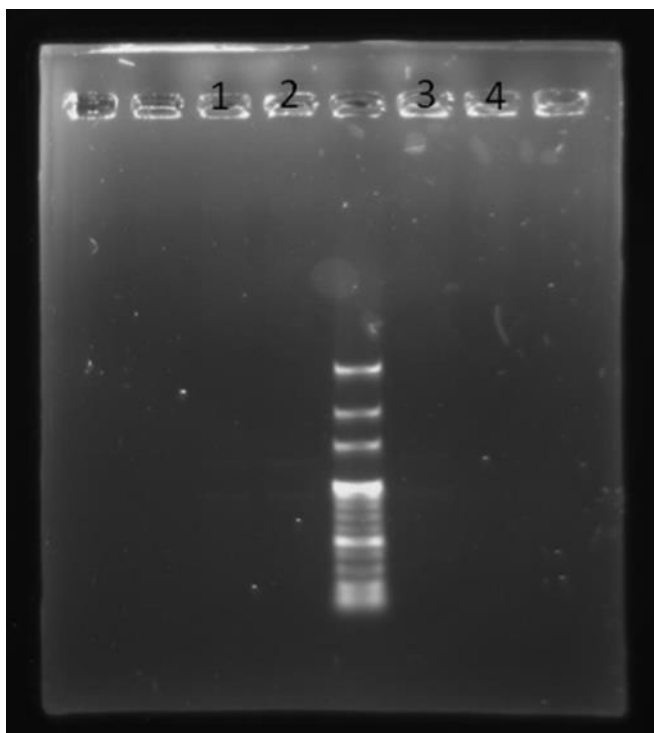
406 bp



2014/9/18

1. Digestion check B0012+B0012

Xho1 Digestion: 892bp & 1268bp



2014/9/22

1. Construct2, B0012, B0015, B0012+B0012 digestion onst
EcoR1 and Spe1 digestion

2014/9/23

1. Gel extraction

Sample	ng/ λ	260/280
B0012(ES)	31.78	0.74
B0012+B0012(ES)	28.04	0.71
B0015(ES)	31.14	0.74
Construct2 (ES)	19.30	0.56

2. Ligation

- (1) Construct 2+B0012
- (2) Construct 2+B0015
- (3) Construct 2+ B0012+B0012

2014/9/24

1. Transforamtion

- (1) Construct 2+B0012
- (2) Construct 2+B0015
- (3) Construct 2+B0012+B0012

2014/9/25

1. Ligation

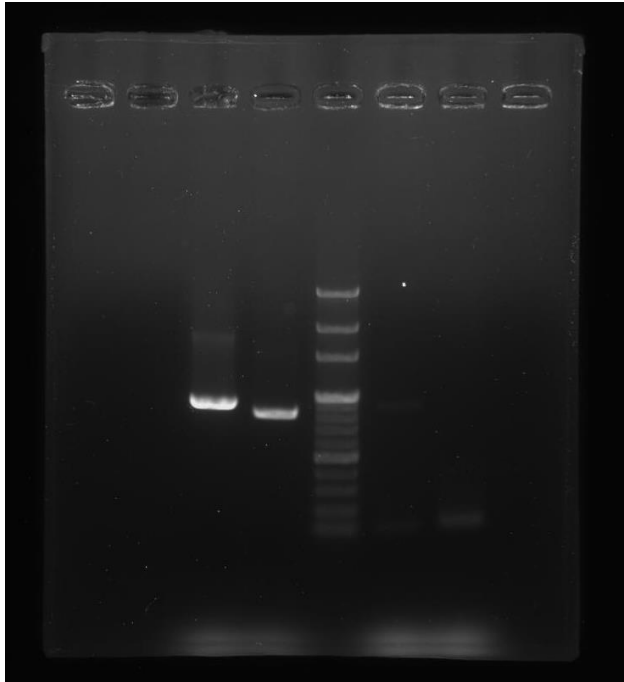
- (1) Construct 2+B0012
- (2) Construct 2+B0015
- (3) Construct 2+ B0012+B0012

2. Transformation

- (1) Construct 2+B0012
- (2) Construct 2+B0015
- (3) Construct 2+B0012+B0012

3. Plasmid PCR (Construct 1_15 & Construct 2_5)

Result= OK



2014/9/26

1. Colony PCR from construct1 & construct2
2. Ligation & Transformation
 - (1) Construct 2 + B0012 + B0012
 - (2) Construct 2 + B0012
 - (3) Construct 2 + B0015

2014/9/27

1. Colony PCR
 - (1) Construct2 + B0015
 - (2) Construct2 + B0012x2
2. Part submission PCR
 - (1) Constitutive promoter
 - (2) nlmC promoter
 - (3) constitutive promoter + GFP
 - (4) nlmC promoter + GFP
3. electrophoresis
4. KOD PCR
 - (1) Constitutive promoter
 - (2) nlmC promoter
 - (3) constitutive promoter + GFP
 - (4) nlmC promoter + GFP
5. Transformation
 - (1) Construct2 + B0012

- (2) Construct2 +B0015
- (3) Construct2 +B0012+B0012

2014/9/28

1. Electrophoresis of KOD PCR of 2014/9/27
2. KOD PCR
 - (1) Constitutive promoter+GFP
 - (2) nlmC promoter+GFP
3. electrophoresis of KOD PCR of 2014/9/28
4. plasmid extraction

Sample	ng/ λ	260/280
PnlmC KOD	54.22	0.71
Pcon KOD	54.25	0.65
Construct1_1	79.54	0.84
Construct1_2	93.97	0.90
Construct1_3	104.30	0.97
Construct1_4	81.94	0.84
Construct1_5	63.38	0.71
Construct1_6	102.13	0.89
Construct2_1	92.82	0.88
Construct2_2	89.00	0.87
Construct2_3	104.13	0.92
Construct2_4	89.75	0.87
Construct2_5	79.34	0.88
Construct2_6	69.18	0.76
Construct2_B0012+B0012_1	78.79	0.81
Construct2_B0012+B0012_2	70.37	0.76
Construct2_B0012+B0012_3	85.11	0.87
Construct2_B0015_2	84.63	0.86
Construct2_B0015_3	82.87	0.83

2014/9/29

1. plasmid PCR
 - (1) construct1
 - (2) construct2
 - (3) construct2+B0012+B0012
 - (4) construct2+B0015
2. electrophoresis of constitutive promoter (KOD)
3. Ligation for part submission
 - (1) E1010+PnlmC

(2) E1010+Pcon

4. gel extraction

Sample	ng/ λ	260/280
Pcon-1	53.93	0.66
Pcon-2	48.43	0.63
Pcon-3	48.78	0.62

5. digestion

(1) Pcon-1

(2) Pcon-2

(3) Pcon-3

6. Transformation

(1) E1010+PnlmC

(2) E1010+Pcon

2014/10/4

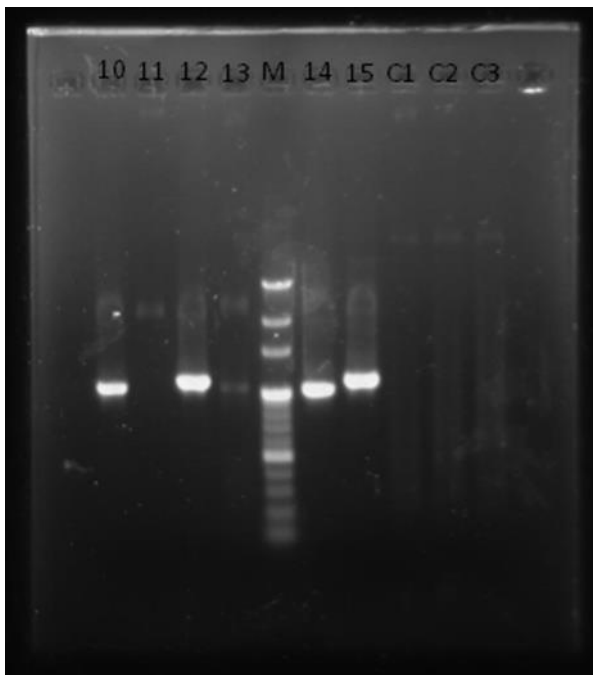
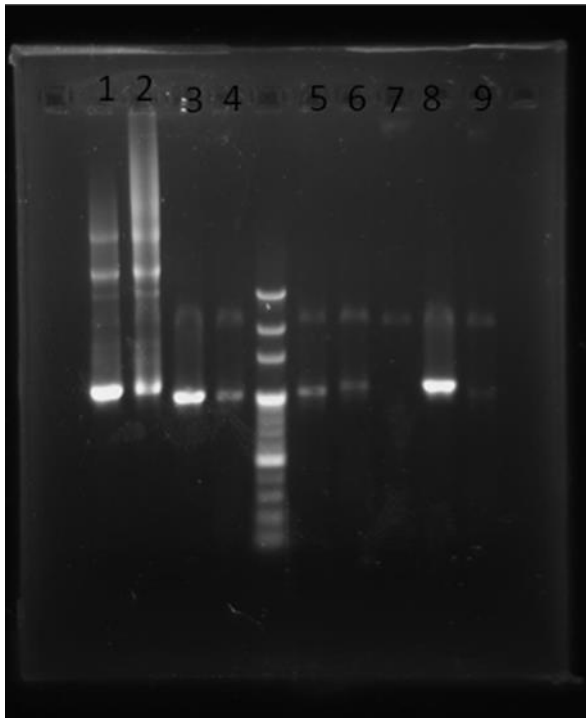
1. Plasmid extraction of Pcon+GFP pSB1C3

Sample	ng/ λ	260/280
Pcon+GFP_pSB1C3_1	114.29	1.52
Pcon+GFP_pSB1C3_2	98.60	1.57
Pcon+GFP_pSB1C3_3	101.28	1.63
Pcon+GFP_pSB1C3_4	132.84	1.61
Pcon+GFP_pSB1C3_5	119.00	1.64
Pcon+GFP_pSB1C3_6	116.63	1.52
Pcon+GFP_pSB1C3_7	90.64	1.53
Pcon+GFP_pSB1C3_8	119.83	1.61
Pcon+GFP_pSB1C3_9	119.90	1.64
Pcon+GFP_pSB1C3_10	109.62	1.64
Pcon+GFP_pSB1C3_11	68.73	1.49
Pcon+GFP_pSB1C3_12	81.81	1.51
Pcon+GFP_pSB1C3_13	95.97	1.61
Pcon+GFP_pSB1C3_14	106.68	1.63
Pcon+GFP_pSB1C3_15	107.78	1.06

2. Plasmid PCR of Pcon_GFP_pSB1C3

2014/10/5

1. Electrophoresis of Pcon_GFP_pSB1C3



2. Digestion of Construct 2 (EcoR1, Spe1)

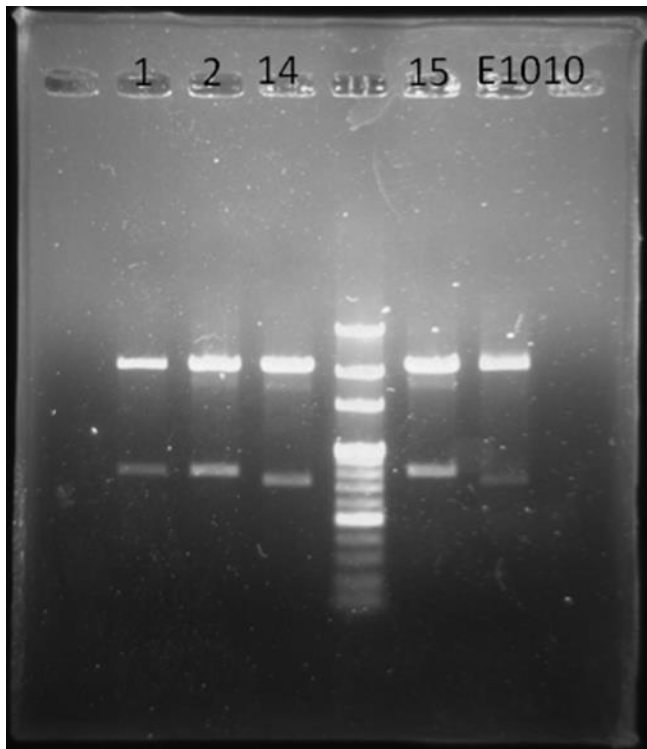
3. Gel extraction of Construct 2 (EcoR1, Spe1)

Sample	ng/ λ	260/280
Construct 2 E.S.	22.28	0.90

4. Ligation of construct 2 and B0012

2014/10/5

1. Digestion check



2. Construct2 digestion without gel extraction, just filter by the column

2014/10/6

1. Transformation: construct 2 + B0012

2. Electrophoresis

(1) Construct1

(2) Construct2

(3) Construct2+B0012+B0012

(4) Construct2+B0015

3. Digestion check

(1) PnlmC

(2) Pcon

4. Colony PCR

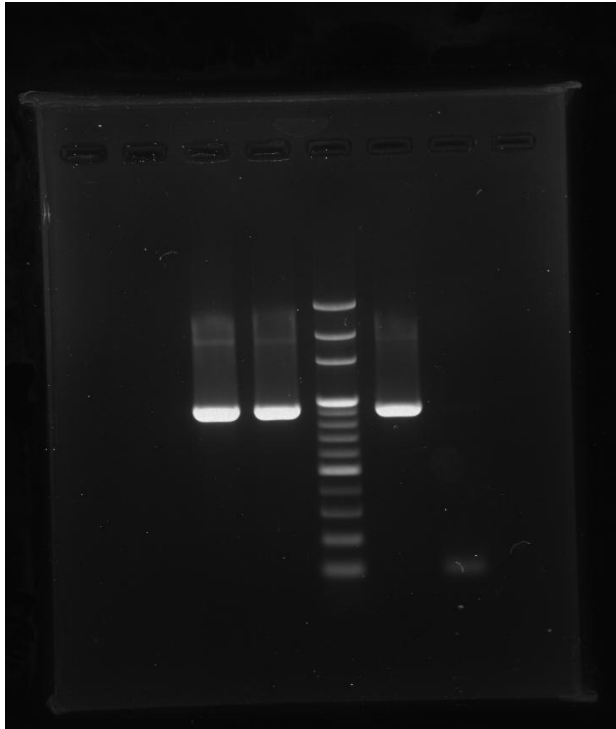
(1) Construct2 +B0015

5. Plasmid PCR

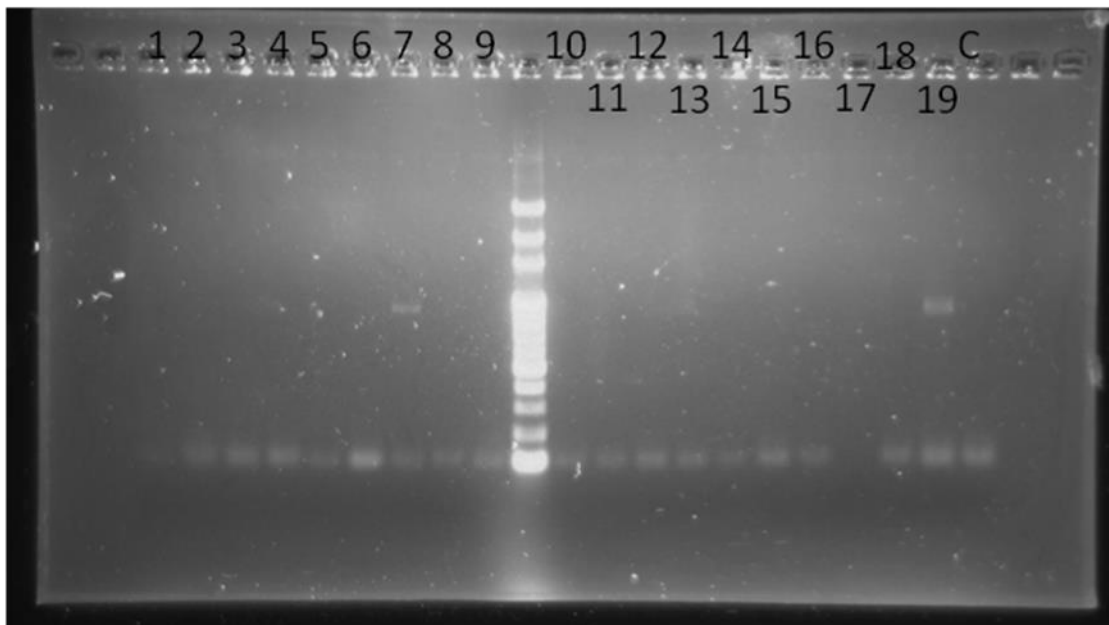
(1) Construct2 in S.mutans

2014/10/7

1. Electrophoresis of construct 2 in S.mutans



2. Colony PCR of construct 2 +B0012
3. Electrophoresis of colony PCR of construct 2+B0012



2014/10/8

1. Transformation of *S. mutans*
 - (1) Construct1
 - (2) Construct2 +B0012
 - (3) Construct2+B0015

2014/10/9

1. Plasmid extraction

Sample	ng/ λ	260/280
Construct2+B0012_2	62.24	1.53
Construct2+B0012_17	75.98	1.60
Construct2+B0012_19	80.76	1.61
Construct 1 in S.mutans	12.05	0.70

2. Ligation

(1) Construct2 +B0015

3. Plasmid PCR

(1) Construct1

(2) Construct2+B0012

4. Electrophoresis

1= construct2 +B0012_2 using new dye

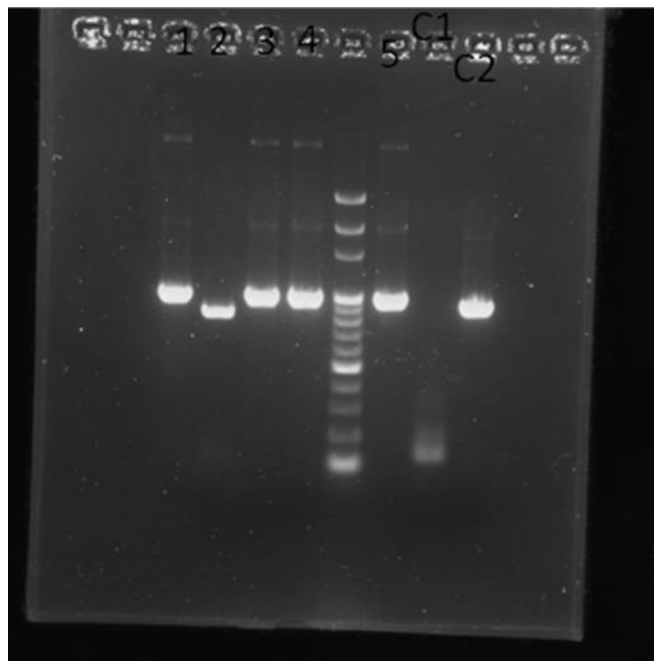
2= construct1 in S.mutans

3=construct2+B0012_2

4=construct2+B0012_17

5=construct2+B0012_19

C1, C2=negative control



2014/10/10

1. Transformation of S.mutans(construct2 +B0012)

2. Transformation(construct2+B0015)

3. Ligation(construct2+B0015)

2014/10/11

1. Plasmid extraction

Sample	ng/ λ	260/280
Construct2+B0015_1	13.29	0.79
Construct1_3	12.01	0.76
Construct2+B0015_2	14.54	0.82
Construct2+B0012+B0012_4	23.26	1.11
Construct1_4	14.68	0.80
Construct2+B0012+B0012_3	12.63	0.79

2. Plasmid PCR= failed

2014/10/12

1. Colony PCR of construct2 +B0015

2. Electrophoresis

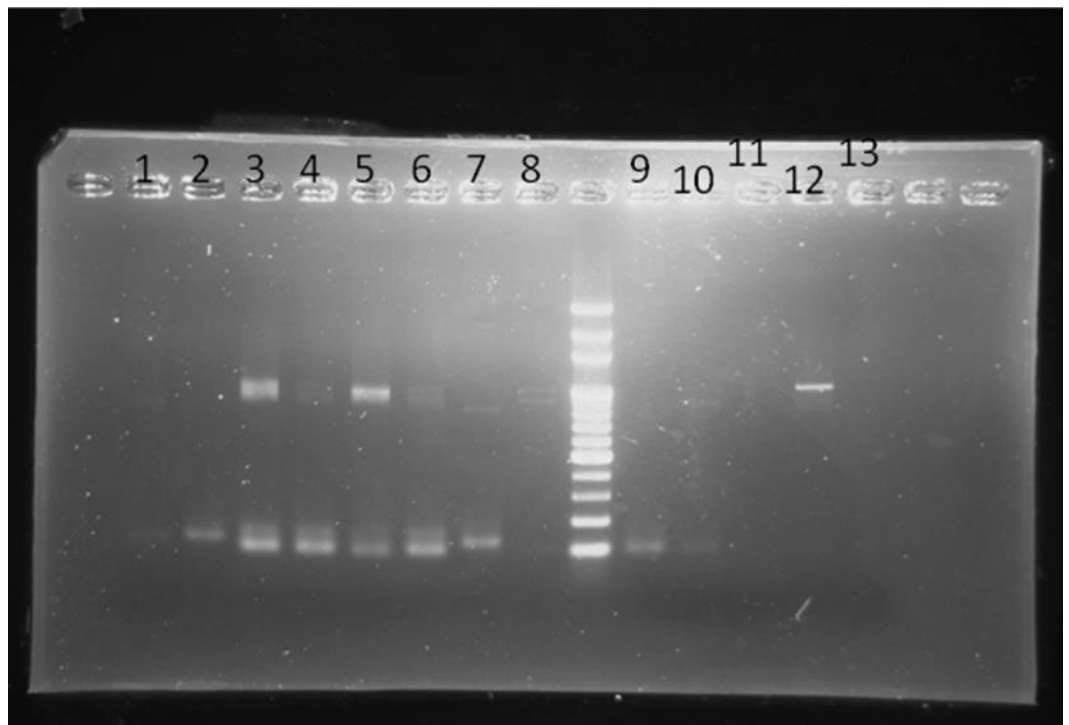
1,2= construct 1(S.mutans)

3,4= construct 2 +B0015(S.mutans)

5,6= construct 2 +B0012x2(S.mutans)

7,8= control

9~13= construct 2 +B0015 colony PCR (E.coli)



2014/10/13

1. Plasmid extraction

Sample	ng/ λ	260/280
Construct2+B0012x2_1	18.94	0.93

Construct2+B0012x2_2	19.78	0.99
Construct1_1	19.60	0.90
Construct1_4	15.13	0.90
Construct2+B0015_3	21.98	1.05
Construct2+B0015_4	20.12	1.06
Construct2+B0012_1	20.43	0.96
Construct2+B0012_2	37.39	1.26
Construct2+B0012_3	15.88	0.90
Construct2+B0012_4	21.88	1.02
Construct2+B0012_5	17.04	0.84
Construct2+B0012_6	22.85	1.06
Construct2+B0012_7	21.06	1.01
Construct2+B0012_8	19.82	0.96
Construct2+B0012_9	21.09	0.99

2014/10/14

1. Electrophoresis of plasmid PCR(Construct1, Construct2+B0012x2, Construct2+B0015)= fail